PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Glen H. ERIKSON et al.

Serial No: 09/664,827 Group Art Unit: 1655

Filed: September 19, 2000 Examiner: C. Wilder

Att. Docket No.: E1047/20044 Confirmation No.:

For: QUADRUPLEX DNA AND DUPLEX PROBE SYSTEMS

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington, DC 20231

Sir:

I, Jasmine I. Daksis, Ph.D., a citizen of Canada, hereby declare and state:

1. The curriculum vitae attached as Exhibit A accurately reflects my professional credentials.

2. I am a joint inventor named in the above-identified application, as well as the Senior Scientist of Ingeneus Research, an affiliate of the assignee of the application, Ingeneus Corp. As such, I am familiar with the application and the correspondence with the United States Patent and Trademark Office relating to the application.

3. This Declaration is submitted at the suggestion of Examiner Jeffrey Fredman of the United States Patent and Trademark Office, who asked me to present evidence showing that the novel binding motifs of the invention are not merely misinterpreted examples of previously known strand invasion binding motifs. While the claims at issue in this application specify that the multiplex comprises four strands, Examiner Fredman indicated at the January 15, 2002 interview that he would be satisfied with a showing based on triplex binding studies, which were readily available for use in this

context, based on their previous use in other contexts. The triplex binding studies described below were previously conducted by me to show that hybridization of non-denatured dsDNA targets and ssDNA probes in the presence of YOYO-1 is not based on D-loop formation.

4. It was Examiner Fredman's position at the interview that binding reactions under non-denaturing conditions implied that either novel dsDNA:ssDNA triple strand structures were forming, or that localized strand invasion and displacement by the ssDNA probe had occurred, resulting in D-loop formation in the duplex DNA target. It is well known that a PNA probe may cause localized separation of duplex strands of a dsDNA target before binding in duplex form to the complementary base sequence in the target, forming a stable D-loop consisting of a localized ssPNA:ssDNA:ssPNA triplex and a displaced ssDNA strand. See, e.g., Tomac et al., "Ionic Effects on the Stability and Conformation of Peptide Nucleic Acid Complexes." J. Am. Chem. Soc. 118, 5544-5552 (1996) (attached to the concurrently filed Information Disclosure Statement). A P-loop structure is also known to be possible, PNA:DNA:PNA, after strand displacement. The strand invasion process can be inhibited by the addition of increasing NaCl concentrations, which causes the stability of the duplex dsDNA target to be increased to inhibit separation of the duplex dsDNA strands, thereby preventing strand invasion and D-loop or P-loop formation by a PNA strand. See, e.g., Tomac et al. Increasing NaCl concentrations should yet more readily and completely inhibit D-loop strand displacement and invasion by a ssDNA oligonucleotide. Moreover, strand invasion is a phenomenon only reported in association with nucleobase-containing sequences having uncharged or partially charged backbones (i.e., PNA or the like).

5. To determine whether the triple strand recognition and binding depended on D-loop formation as a result of strand displacement, wild-type non-denatured 50-mer dsDNA target (SEQ ID NO:6 of the Table attached as Exhibit B) was reacted at room temperature with equimolar amounts of the 15-mer antiparallel ssDNA Probe No. 2 (see Exhibit B) in the presence of 500 nM YOYO-1, 10 mM Tris, pH 7.5, 1 mM EDTA and various NaCl concentrations ranging from 0 to 150 mM NaCl. As illustrated in the figure attached as Exhibit C, increased NaCl concentrations not only inhibited displacement of the duplex strands of the dsDNA target by the ssDNA probe, preventing D-loop formation, they actually promoted perfectly matched dsDNA:ssDNA triple strand formation at room temperature, as evidenced by an observed increase in fluorescence. Fluorescence was provided by the intercalation of YOYO-1 into the multiplex structure. YOYO-1 is well known to stabilize all nucleic acid duplexes and raise their Tm. DNA triple strand formations exhibited a much higher level of fluorescent emission than the DNA duplex control in the presence of YOYO-1.

6. Incompletely complementary triple strand complexes containing a 1 bp T-G mismatch (SEQ ID NO:8 + Probe No. 2) produced fluorescent emission intensities that were 59% lower than those observed with the perfectly matched complexes in the absence (Exhibit C) or presence of NaCl (data not shown).

7. Further confirmation that triple strand complexes were formed in the presence of YOYO-1 was obtained by HPLC analyses carried out in our lab under my supervision. Triple strand complexes, consisting of 15-mer ssDNA Probe No. 2 bound to wild-typ non-denatured 50-mer dsDNA target (SEQ ID NO:6) and YOYO-1, were clearly separated by HPLC from 50-mer dsDNA target

sequences (SEQ ID NO:6) containing incorporated YOYO-1 but no bound Probe No. 2 (data not shown). Approximately 52 percent of the 50-mer dsDNA target sequences in the sample formed complexes with the 15-mer ssDNA probe and YOYO-1, which were sufficiently stable to withstand purification by HPLC. As expected, the HPLC purified triple strand dsDNA:ssDNA complexes containing YOYO-1 when subsequently lased emitted a higher level of fluorescence than did the HPLC purified non-bound dsDNA containing YOYO-1, following irradiation with the laser (data not shown).

8. Thus, the claimed multiplexes, like the triplexes discussed above, are not formed through a strand displacement mechanism, but rather are novel specifically bound complexes wherein at least one nucleobase binds specifically to two other nucleobases.

9. Further evidence that the multiplexes formed in accordance with the invention are Watson-Crick based is provided in the form of Exhibit D. Exhibit D uses three of the complexes formed in Example 4 of the application to show how the data suggest a Watson-Crick based quadruplex, rather than a theoretical construct of purine motif quadruplex binding (extrapolated from the purine motif binding known to occur in triplexes that are stable at physiological pH). Assuming that the theoretical construct obeys the same base pairing rules as the purine triplex motif, the number of mismatches (4 to 5 per 15-mer probe strand) in the purine motif makes it very unlikely that any quadruplexes would have been formed or detected. Moreover, Fig. 4 of the application shows that the combination of Target A + Probe C has a greater fluorescent intensity than either of the other two combinations analyzed in Exhibit D. Since the intensity for the tests done in Example 4 should be inversely related to the number of mismatches, the data is more consistent

with a Watson-Crick binding motif (where Targ t A + Probe C has the lowest number of mismatches, 0 vs. 1) than with the purine binding motif.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: January 18, 2002

Jasmine Daksis

Jasmine I. Daksis, Ph.D.

CURRICULUM VITAE

Personal Details

Name: **JASMINE I. DAKSIS**

Home Address: 36 Cartier Crescent
Richmond Hill, Ontario
Canada L4C 2N2

Telephone: (905) 883-9467

Birthplace: Toronto, Canada
Citizenship: Canadian

Education

Degree	Date	Institution	Department	Supervisor
B.Sc.(with distinction)	April 1978	University of Toronto	Biochemistry	
M.Sc.	July 1981	University of Toronto	Microbiology	Dr. V.L. Chan
Ph.D.	Sept. 1987	University of Toronto	Microbiology	Dr. V.L. Chan

M.Sc. Thesis: Preliminary characterization of temperature-sensitive mutants of herpes simplex virus type 1

Ph.D. Thesis: Biochemical and genetic characterization of temperature-sensitive mutants of herpes simplex virus type 1 defective in the shutoff of cellular macromolecular synthesis

Graduate Awards

Sept. 1978 - Sept. 1980	University of Toronto Open Fellowship
May 1981 - April 1982	Cancer Research Society Inc. Fellowship
April 1982 - July 1986	National Cancer Institute of Canada Studentship
July 1986 - Jan. 1987	Life Sciences Committee, University of Toronto Research Board Fellowship
Oct. 1987	L.W. Macpherson Microbiology Award

Postgraduate Awards

Oct. 1987 - July 1988	European Molecular Biology Organization Long Term Fellowship
July 1988 - July 1991	National Cancer Institute of Canada Fellowship
June 1992 - June 1994	The Hospital for Sick Children Restracom Postdoctoral Fellowship

Previous Positions Held

Research Experience

Position	Date	Institution	Supervisor
Senior Scientist	May/98 - present	Ingeneus Research	
Manager, Scientific Communications	June/96 - Aug/96	Karisma Communications	
Research Associate	Sept/94 - Sept/95	Div. of Cancer Biology Research Sunnybrook Health Science Center	Dr. J. Slingerland
Research Project:	Mechanisms of resistance to growth inhibition by TGF-β in human breast epithelial cells		
Post-doctoral Fellow	Mar/92 - Aug/94	Departments of Microbiology, Immunology & Cancer Research The Hospital for Sick Children	Dr. L.J.Z. Penn
Research Project:	Myc induction of cyclin D1 transcription and cell cycle progression		
Post-doctoral Fellow	Oct/87 - Nov/91	MRC Virology Unit Institute of Virology, Glasgow, UK	Dr. C.M. Preston
Research Project:	VP16 activation of HSV immediate early gene transcription during the cell cycle		
Ph.D. Student	Sept/81 - Sept/87	Department of Microbiology University of Toronto	Dr. R.V.L. Chan
M.Sc. Student	Sept/78 - July/81	Department of Microbiology University of Toronto	Dr. R.V.L. Chan
Summer Student & Project Student	May/77 - Apr/78	Department of Biochemistry University of Toronto	Dr. J.T. Wong

Teaching Experience

Teaching Assistant	Sept/78 - Apr/80	University of Toronto

Elected Offices - Department of Microbiology, University of Toronto

Secretary/Treasurer - Graduate Student Union	Sept/78 - Sept/79

Vice-president - Graduate Student Union	Sept/79 - Sept/80
Student Rep. for Departmental Staff Meetings	Sept/80 - Sept/83
Dept. Rep. for Grad. Org. of the Faculty of Medicine	Sept/85 - Sept/86
Organizer for Dept. Molecular Biology Journal Club	Sept/82 - Sept/87

Publications

1. **Daksis, J.I.**, M.M. Priemer and V.L. Chan. (1982). Isolation and preliminary characterization of a phosphonoacetic acid-resistant and temperature-sensitive mutant of herpes simplex virus type 1. J. Virol. 42:20-29.

2. **Daksis, J.I.**, J.M. Cocking, M.A. Skinner and V.L. Chan. (1987). Temperature-sensitive herpes simplex virus type 1 mutants defective in the shutoff of cellular DNA synthesis and host polypeptide synthesis. Virus Res. 6:297-315.

3. **Daksis, J.I.** and V.L. Chan. (1987). Physical mapping of two herpes simplex virus type 1 host shutoff loci: rescue of each *ts* mutation occurs with two unique cloned regions of the viral genome. J. Virol. 61:143-150.

4. Arko, E.F., **J.I. Daksis** and V.L. Chan. (1991). Further characterization of *ts*1-8, a mutant of herpes simplex virus type 1. Virus Res. 20:71-83.

5. McFarlane, M., **J.I. Daksis** and C.M. Preston. (1992). Hexamethylene bisacetamide stimulates herpes simplex virus immediate early gene expression in the absence of trans-induction by Vmw65. J. Gen. Virol. 73:285-292.

6. **Daksis, J.I.** and C.M. Preston. (1992). Herpes simplex virus immediate early gene expression in the absence of transinduction by Vmw65 varies during the cell cycle. Virology 189:196-202.

7. **Daksis, J.I.**, R.Y. Lu, L.M. Facchini, W.W. Marhin and L.J.Z. Penn. (1994). Myc induces cyclin D1 expression in the absence of *de novo* protein synthesis and links mitogen-stimulated signal transduction to the cell cycle. Oncogene 9:3635-3645.

8. Jamieson, D.R.S., L.H. Robinson, **J.I. Daksis**, M.J. Nicholl and C.M. Preston. (1995). Quiescent viral genomes in human fibroblasts after infection with herpes simplex virus type 1 Vmw65 mutants. J. Gen. Virol. 76:1417-1431.

9. C. Sandhu, J. Garbe, N. Bhattacharya, **J.I. Daksis**, C.-H. Pan, P. Yaswen, J. Koh, J.M. Slingerland, and M.R. Stampfer. (1997). Transforming growth factor β stabilizes $p15^{INK4B}$ protein, increases $p15^{INK4B}$-cdk4 complexes, and inhibits cyclin D1-cdk4 association in human mammary epithelial cells. Mol. Cell. Biol. 17:2458-2467.

Manuscripts in Preparation

1. Lu, R.Y., **J.I. Daksis,** and L.J.Z. Penn. (1996). Subtractive cloning identifies a heat-shock gene as a Myc-induced cDNA. (To be submitted to Mol. Cell. Biol.)

2. Marhin, W., L. Facchini, **J.I. Daksis**, V. Savova and L.J.Z. Penn. (1996). Platelet derived growth factor β receptor expression is suppressed by the *myc* oncogene.

Abstracts

1. **Daksis, J.I.**, M.M. Priemer and V.L. Chan. (1981). Isolation and preliminary characterization of a phosphonoacetic acid-resistant and temperature-sensitive mutant of herpes simplex virus type 1. In "The Canadian Federation of Biological Sciences", Montreal, Canada.

2. **Daksis, J.I.** and V.L. Chan. (1981). Temperature-sensitive mutants of herpes simplex virus type 1 defective in the shutdown of cellular DNA synthesis. In "International Workshop on Herpesviruses", Bologna, Italy.

3. **Daksis, J.I.**, M.M. Priemer and V.L. Chan. (1981). A phosphonoacetic acid-resistant and temperature-sensitive mutant of herpes simplex virus type 1. In "Fifth International Congress of Virology", Strasbourg, France.

4. **Daksis, J.I.**, J.M. Cocking and V.L. Chan. (1984). Temperature-sensitive herpes simplex virus type 1 mutants defective in the shutoff of cellular DNA synthesis are also defective in the secondary suppression of host polypeptide synthesis. In "Ninth International Herpesvirus Workshop", Seattle, Washington, USA.

5. **Daksis, J.I.** and V.L. Chan. (1984). Physical mapping of two HSV-1 *ts* mutants defective in the shutoff of cellular macromolecular synthesis: rescue of each mutant occurs with two unique cloned regions of the HSV-1 genome. In "Ninth International Herpesvirus Workshop", Seattle, Washington, USA.

6. **Daksis, J.I.** and V.L. Chan. (1986). Physical mapping of two HSV-1 host shutoff loci: rescue of each *ts* mutation occurs with two unique cloned regions of the viral genome. In "Canadian Society of Microbiologists", Toronto, Canada.

7. **Daksis, J.I.** and V.L. Chan. (1986). Two unique cloned regions of the HSV-1 genome are able to rescue *ts* mutants defective in host shutoff. In "Eleventh International Herpesvirus Workshop", Leeds, England.

8. **Daksis, J.I.** and C.M. Preston. (1991). HSV immediate early transcription is cell cycle specific in the absence of Vmw65. In "Sixteenth International Herpesvirus Workshop", Pacific Grove, California, USA.

9. McFarlane, M., **J.I. Daksis**, M.J. Nicholl and C.M. Preston. (1991). Differentiation-inducing agents activate HSV immediate early transcription. In "Sixteenth International Herpesvirus Workshop", Pacific Grove, California, USA.

10. Penn, L.J.Z., **J.I. Daksis** and R.Y. Lu. (1993). Subtractive cloning identifies a heat-shock gene as a Myc-induced cDNA. In "Twenty-second Keystone Symposia on Molecular and Cellular Biology", J. Cell. Biochem. Abstract Supplement 17A:139.

11. **Daksis, J.I.**, R.Y. Lu and L.J.Z. Penn. (1993). Myc induces cyclin D1 transcription and G1 to S phase progression of the cell cycle. In "Gordon Conference: Molecular and Genetic Basis of Cellular Proliferation", Meriden, New Hampshire, USA.

12. Marhin, W., L. Facchini, **J.I. Daksis**, V. Savova and L.J.Z. Penn. (1993). Platelet derived growth factor β receptor expression is suppressed by the *myc* oncogene. In "Ninth Annual Meeting on Oncogenes", Frederick, Maryland, USA.

13. **Daksis, J.I.**, R.Y. Lu and L.J.Z. Penn. (1993). Myc induces cyclin D1 transcription and G1 to S phase progression of the cell cycle. In "CSH Meeting on Cancer Cells: Mechanisms of Eukaryotic Transcription", Cold Spring Harbor, New York, USA.

14. Stampfer, M., J. Garbe, **J.I. Daksis**, N. Bhattacharya, C. Pan, J. Hosoda, P. Yaswen and J. Slingerland. (1995). Resistance to TGF-β mediated G1 arrest in human mammary epithelial cells: evidence for altered cdk inhibitor activity. In "Keystone Symposia on Molecular and Cellular Biology: Oncogenes: 20 Years Later", Keystone, Colorado, USA.

15. Slingerland, J., J. Garbe, C.-H. Pan, J. Hosoda, **J.I. Daksis**, and M. Stampfer. (1995). Mechanisms of resistance to transforming growth factor-beta (TGF-β) induced cell cycle arrest in human mammary epithelial cells. In "American Association for Cancer Research Meeting", Toronto, Ontario, Canada.

16. Wasfy, G., W. Marhin, R. Lu, **J.I. Daksis** and L.J.Z. Penn. (1995). Cloning and identification of Myc-regulated genes. In "American Association for Cancer Research Meeting", Toronto, Ontario, Canada.

DAKSIS, Jasmine I.

Graduate Courses

Major and minor credits in the Department of Microbiology, University of Toronto. Outside minor credit in the Department of Medical Genetics, University of Toronto.

Major:	Recent Advances in Microbiology (MPL 1131L)		(Final Grade = A)
	Dr. R. Sheinin	"Eukaryotic DNA Replication: Cellular and Viral"	(A)
	Dr. V.L. Chan	"Genetics of DNA Tumour Viruses"	(A)
Minor:	Advanced Topics in Virology (MPL 1133L)		(Final Grade = A^-)
	Dr. J. Campbell	"Viral Infection: Prophylaxis and Therapy"	
Minor:	Special Topics in Microbiology (MPL 1132L)		(Final Grade = A)
	Dr. A. Guha	"Nucleotide Sequencing and Gene Mapping"	
Outside Minor:	Somatic Cell and Human Genetics (MBP 1014L)		(Final Grade = A)
	Dr. J. Greenblatt	"Transcriptional Regulatory Mechanisms"	(A^+)
	Dr. H. Willard	"Aspects of the Molecular Organization of the Human Genome"	(A^+)
	Dr. B. Williams	"Transcriptional and Translational Controls in Viral and Cellular Genomes"	(A^-)

References

Robert A. Phillips, Ph.D.
Executive Director
National Cancer Institute
of Canada
10 Alcorn Avenue, Suite 200
Toronto, Ontario M4V 3B1

Tel: 416-961-7223
Fax: 416-961-4189

Chris M. Preston, Ph.D.
Senior MRC Staff Member
MRC Virology Unit
Institute of Virology
Church Street
Glasgow G11 5JR
Scotland, U.K.

Tel: 011-44-141-339-8855, ext 4635 or
011-44-141-330-4630 or 011-44-141-330-3921
Fax: 011-44-141-337-2236

Linda J.Z. Penn, Ph.D.
Senior Scientist
Division of Cellular and
Molecular Biology
Ontario Cancer Institute
610 University Avenue
Toronto, Ontario M5G 2C1

Tel: 416-946-2276
Fax: 416-946-2065

Howard S. Marsden, Ph.D.
Senior MRC Staff Member
MRC Virology Unit
Institute of Virology
Church Street
Glasgow G11 5JR
Scotland, U.K.

Tel: 011-44-141-339-8855, ext 4028 or 4639
Fax: 011-44-141-337-2236

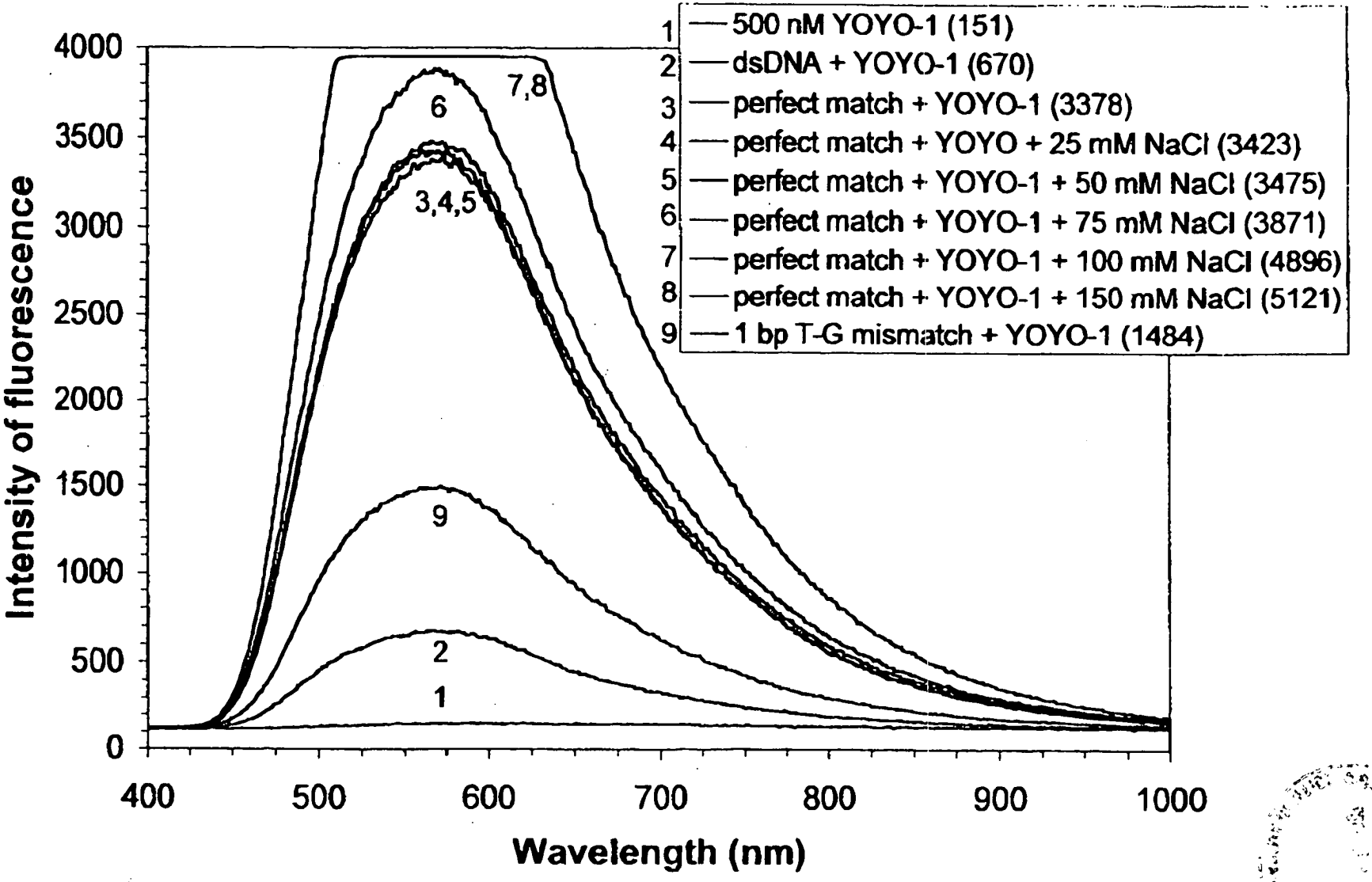
TABLE 1.

SEQ ID NO.	TARGET SEQUENCES	% GC
1	5'-TGGCACCATTAAAGAAA **ATATCATCTTTGGTG** TTTCCTATGATGAATATA-3' 3'-ACCGTGGTAATTTCTTT **TATAGTAGAAACCAC** AAAGGATACTACTTATAT-5'	30%
2	5'-TGGCACCATTAAAGAAA **ATATCGTCTTTGGTG** TTTCCTATGATGAATATA-3' 3'-ACCGTGGTAATTTCTTT **TATAGCAGAAACCAC** AAAGGATACTACTTATAT-5'	32%
3	5'-TGGCACCATTAAAGAAA **ATATACTCTTTGGTG** TTTCCTATGATGAATATA-3' 3'-ACCGTGGTAATTTCTTT **TATATGAGAAACCAC** AAAGGATACTACTTATAT-5'	30%
4	5'-TGGCACCATTAAAGAAA **ATATACGCTTTGGTG** TTTCCTATGATGAATATA-3' 3'-ACCGTGGTAATTTCTTT **TATATGCGAAACCAC** AAAGGATACTACTTATAT-5'	32%
5	5'-TGGCACCATTAAAGAAA **ATATCAT ... TGGTG** TTTCCTATGATGAATATA-3' 3'-ACCGTGGTAATTTCTTT **TATAGTA ... ACCAC** AAAGGATACTACTTATAT-5'	28%
6	5'-GAGCACCATGACAGACA **CTGTCATCTCTGGTG** TGTCCTACGATGACTCTG-3' 3'- CTCGTGGTACTGTCTGT **GACAGTAGAGACCACACAGGATGCTACTGAGAC-5'**	52%
7	5'-GAGCACCATGACAGACA **CTGTCGTCTCTGGTG** TGTCCTACGATGACTCTG-3' 3'- CTCGTGGTACTGTCTGT **GACAGCAGAGACCACACAGGATGCTACTGAGAC-5'**	54%
8	5'-GAGCACCATGACAGACA **CTGTCATCTTTGGTG** TGTCCTACGATGACTCTG-3' 3'- CTCGTGGTACTGTCTGT **GACAGTAGAAACCACACAGGATGCTACTGAGAC-5'**	50%
9	5'-GAGCACCATGACAGACA **CTGTACTCTCTGGTG** TGTCCTACGATGACTCTG-3' 3'- CTCGTGGTACTGTCTGT **GACATGAGAGACCACACAGGATGCTACTGAGAC-5'**	52%

10	5'- GAGCACCCTCCCAGGCA **CGGTCGTCCCTGGTG** CGACCTCCGACGAGCGTG-3' 3'- CTCGTGGGAGGGTCCGT **GCCAGCAGGGACCAC** GCTGGAGGCTGCTCGCAC-5'	72%
11	5'- GAGCACCCTCCCAGGCA **CGGTCATCCCTGGTG** CGACCTCCGACGAGCGTG-3' 3'- CTCGTGGGAGGGTCCGT **GCCAGTAGGGACCAC** GCTGGAGGCTGCTCGCAC-5'	70%
12	5'- GAGCACCCTCCCAGGCA **CGGTATTCCCTGGTG** CGACCTCCGACGAGCGTG-3' 3'- CTCGTGGGAGGGTCCGT **GCCATAAGGGACCAC** GCTGGAGGCTGCTCGCAC-5'	68%

PROBE NO.	DNA PROBES	
1	5'-**CACCAAAGATGATAT**-3'	33%
2	5'-**CACCAGAGATGACAG**-3'	53%
3	5'-**CACCAGGGACGACCG**-3'	73%
4	5'-**CACCAAAGAAGATAT**-3'	33%
5	5'-**CACGAAAGATGATAT**-3'	33%
6	5'-**CACCAAACATGATAT**-3'	33%
7	5'-**CACCATAGATGATAT**-3'	33%
8	5'-**CACCAGAGATGATAT**-3'	40%
9	5'-**CACCACAGATGATAT**-3'	40%
10	5'-**CACCAAAGACGATAT**-3'	40%

11	5'-CACCAAAAATGATAT-3'	27%
12	5'-CACAAAAGATGATAT-3'	27%
13	5'-CACCAGCTATGATAT-3'	40%
14	5'- ATATCATCTTTGGTG -3'	33%
15	5'- ATATCTTCTTTGGTG -3'	33%
16	5'- ATATCATCTTTCGTG -3'	33%
17	5'- ATATCATGTTTGGTG -3'	33%
18	5'- ATATCATCTATGGTG -3'	33%
19	5'- ATATCATCTCTGGTG -3'	40%
20	5'- ATATCATCTGTGGTG -3'	40%
21	5'- ATATCGTCTTTGGTG -3'	40%
22	5'- ATATCATTTTTGGTG -3'	27%
23	5'- ATATCATCTTTTGTG -3'	27%


1 — 500 nM YOYO-1 (151)
2 — dsDNA + YOYO-1 (670)
3 — perfect match + YOYO-1 (3378)
4 — perfect match + YOYO + 25 mM NaCl (3423)
5 — perfect match + YOYO-1 + 50 mM NaCl (3475)
6 — perfect match + YOYO-1 + 75 mM NaCl (3871)
7 — perfect match + YOYO-1 + 100 mM NaCl (4896)
8 — perfect match + YOYO-1 + 150 mM NaCl (5121)
9 — 1 bp T-G mismatch + YOYO-1 (1484)
Intensity of fluorescence
Wavelength (nm)
4000
3500
3000
2500
2000
1500
1000
500
0
400
500
600
700
800
900
1000
7,8
6
3,4,5
9
2
1

Comparison of Watson-Crick Quadruplex vs. Purine Motif Quadruplex

Example 4/Fig. 4

```
Target A + Probe C                                                                  Total No. of
Nested perfectly complementary W-C w/parallel strands 2 and 4.                      Mismatches
Watson-Crick Motif Mismatches                                                           0
Strand 3                                    3'-CTGTCATCTCTGGTG-5'
Strand 4                                    5'-GACAGTAGAGACCAC-3'
Strand 2                  5'-GAGCACCATGACAGACACTGTCATCTCTGGTGTGTCCTACGATGACTCTG-3'
Strand 1                  3'-CTCGTGGTACTGTCTGTGACAGTAGAGACCACACAGGATGCTACTGAGAC-5'
Strand 4                                    5'-GACAGTAGAGACCAC-3'
Strand 3                                    3'-CTGTCATCTCTGGTG-5'
Purine Motif Mismatches*                         X  X     XX X                          5


Target B + Probe C                                                                  Total No. of
1 bp G-T mismatch of partially W-C complementary parallel strands 2 and 4.          Mismatches
Watson-Crick Motif Mismatches                         X                                 1
Strand 3                                    3'-CTGTCATCTCTGGTG-5'
Strand 4                                    5'-GACAGTAGAGACCAC-3'
Strand 2                  5'-GAGCACCATGACAGACACTGTCGTCTCTGGTGTGTCCTACGATGACTCTG-3'
Strand 1                  3'-CTCGTGGTACTGTCTGTGACAGCAGAGACCACACAGGATGCTACTGAGAC-5'
Strand 4                                    5'-GACAGTAGAGACCAC-3'
Strand 3                                    3'-CTGTCATCTCTGGTG-5'
Purine Motif Mismatches                          X       XX X                          4


Target C + Probe C                                                                  Total No. of
1 bp T-T mismatch of partially W-C complementary parallel strands 2 and 4.          Mismatches
Watson-Crick Motif Mismatches                         X                                 1
Strand 3                                    3'-CTGTCATCTCTGGTG-5'
Strand 4                                    5'-GACAGTAGAGACCAC-3'
Strand 2                  5'-GAGCACCATGACAGACACTGTCTTCTCTGGTGTGTCCTACGATGACTCTG-3'
Strand 1                  3'-CTCGTGGTACTGTCTGTGACAGAAGAGACCACACAGGATGCTACTGAGAC-5'
Strand 4                                    5'-GACAGTAGAGACCAC-3'
Strand 3                                    3'-CTGTCATCTCTGGTG-5'
Purine Motif Mismatches                          X       XX X                          4
```

* Purine motif binding known to occur in triplexes that are stable at physiological pH requires that the purine-rich probe strand bind antiparallel to the purine-rich strand of the dsDNA. Assuming that similar rules apply to the theoretical construct of purine motif quadruplex binding (which does not appear to be disclosed in the art), this means that the purine-rich probe strand binds to the antisense strand in these examples. Purine motif binding rules stipulate third strand binding of A to A:T, G to G:C, T to A:T and T to C:G. All other triplex combinations are considered mismatches (noted as "X").